

## IN THE CLAIMS:

Please amend the claims to read as follows:

- 1.-6. (Canceled)
7. (Currently Amended) A method for absolute quantification of a target nucleic acid in a sample comprising the steps of:
- (a) determining the amplification efficiencies of the target nucleic acid and of an internal or external standard under defined amplification conditions ~~as claimed in claim 1;~~ by:
    - (i) preparing a dilution series of the target nucleic acid and a dilution series of the internal or external standard;
    - (ii) amplifying the target nucleic acid and the internal or external standard under defined reaction conditions and measuring the amplification in real-time;
    - (iii) setting a defined signal threshold value;
    - (iv) determining, for each dilution of the target nucleic acid and for each dilution of the internal or external standard, the cycle number at which the signal threshold value is exceeded;
    - (v) determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid and the internal or external standard used for the amplification as a function of the cycle number at which the signal threshold value is exceeded; and
    - (vi) calculating the amplification efficiency of the target nucleic acid and the internal or external standard from said non-linear continuously differentiable function;
  - (b) amplifying the target nucleic acid contained in the sample and of the internal or external standard under ~~the same~~ said defined reaction conditions;
  - (c) measuring the amplification of the target nucleic acid and that of the internal or external standard in real time; and
  - (d) calculating the original copy number in the sample by correcting the copy number derived from step (c) with the amplification efficiencies determined in step (a).

8. (Currently Amended) A method for quantification of a target nucleic acid in a sample relative to a reference nucleic acid comprising the steps of:
- (a) determining the amplification efficiencies of the target nucleic acid and of the reference nucleic acid under defined amplification conditions ~~as claimed in claim 1;~~ by:
    - (i) preparing a dilution series of the target nucleic acid and a dilution series of the reference nucleic acid;
    - (ii) amplifying the target nucleic acid and the reference nucleic acid under defined reaction conditions and measuring the amplification in real-time;
    - (iii) setting a defined signal threshold value;
    - (iv) determining, for each dilution of the target nucleic acid and for each dilution of reference nucleic acid, the cycle number at which the signal threshold value is exceeded;
    - (v) determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid and the reference nucleic acid used for the amplification as a function of the cycle number at which the signal threshold value is exceeded; and
    - (vi) calculating the amplification efficiency of the target nucleic acid and the reference nucleic acid from said non-linear continuously differentiable function;
  - (b) amplifying the target nucleic acid contained in the sample as well as the reference nucleic acid contained in the sample under ~~the same~~ said defined amplification conditions;
  - (c) measuring the amplification of the target nucleic acid and that of the reference nucleic acid in real time; and
  - (d) calculating the original ratio of target nucleic acid and reference nucleic acid in the sample by correcting the ratio derived from step (c) with the amplification efficiencies determined in step (a).
9. (Previously Amended) A method for quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:

- (a) preparing a common or two separate dilution series of target nucleic acid and reference nucleic acid;
- (b) amplifying the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, and measuring the amplification of the nucleic acids in real-time;
- (c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;
- (d) determining the cycle numbers  $C_p$  at which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;
- (e) determining a continuously differentiable function of the  $C_p$  values determined in step d) as a function of the logarithm of the amounts used of target nucleic acid and determining a continuously differentiable function of the  $C_p$  values determined in step d) as a function of the logarithm of the amounts used of reference nucleic acid;
- (f) determining the  $C_p$  values of the target nucleic acid and reference nucleic acid in a sample to be analysed as well as in a calibrator sample;
- (g) assigning the  $C_p$  values measured in step f) to particular values of the functions determined in step e);
- (h) calculating the quotients of the function values from step g) of the target nucleic acid and reference nucleic acid for the sample to be analysed as well as for the calibrator sample; and
- (i) determining the ratio of the two quotients from step h) as a measure of the original amount of target nucleic acid contained in the sample to be analysed.

10. (Previously Amended) A method for quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:

- (a) preparing a common or two separate dilution series of target nucleic acid and reference nucleic acid;
- (b) amplifying the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, and measuring the amplification of the nucleic acids in real-time;

- (c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;
  - (d) determining the cycle numbers  $C_p$  at which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;
  - (e) determining a continuously differentiable function of the logarithm of the amounts used of target nucleic acid as a function of the  $C_p$  values determined in step d) and determining a continuously differentiable function of the logarithm of the amounts used of reference nucleic acid as a function of the  $C_p$  values determined in step d);
  - (f) determining the  $C_p$  values of the target nucleic acid and reference nucleic acid in a sample to be analysed as well as in a calibrator sample;
  - (g) assigning the  $C_p$  values measured in step f) to particular values of the functions determined in step e);
  - (h) calculating the quotients of the function values from step g) of the target nucleic acid and reference nucleic acid for the sample to be analysed as well as for the calibrator sample; and
  - (i) determining the ratio of the two quotients from step h) as a measure of the original amount of target nucleic acid contained in the sample to be analysed.
11. (Previously Amended) The method of claim 10, wherein the continuously differentiable functions from step e) are determined with a polynomial fit.
  12. (Previously Amended) The method of claim 10, wherein the amplified nucleic acids are detected with at least one fluorescently-labeled hybridization probe.
  13. (Previously Amended) The method of claim 12, wherein the amplified nucleic acids are detected with FRET hybridization probes, molecular beacons, or TAQMAN<sup>®</sup> probes.
  14. (Previously Amended) The method of claim 10, wherein the amplified nucleic acids are detected with a DNA-binding dye.

15. (Currently Amended) ~~The method of claim 6, wherein said polynomial fit is of the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> or 7<sup>th</sup> degree~~ A method for determining the efficiency of an amplification of a target nucleic acid comprising the steps of:
- (a) preparing a dilution series of the target nucleic acid;
  - (b) amplifying the target nucleic acid under defined reaction conditions and measuring the amplification in real-time;
  - (c) setting a defined signal threshold value;
  - (d) determining, for each dilution, the cycle number at which the signal threshold value is exceeded;
  - (e) determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid used for the amplification as a function of the cycle number at which the signal threshold value is exceeded, wherein the non-linear continuously differentiable function from step (e) is determined with a polynomial fit of the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> or 7<sup>th</sup> degree; and
  - (f) calculating the amplification efficiency from said non-linear continuously differentiable function.
16. (Previously Added) The method of claim 11, wherein said polynomial fit is of the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> or 7<sup>th</sup> degree.
17. (Previously Added) The method of claim 14, wherein said DNA-binding dye is SYBR<sup>®</sup> Green I.
- 18.-22. (Canceled)
23. (Currently Amended) ~~The method of claim 19,~~ A method for determining amplification efficiency of a nucleic acid as a function of concentration comprising:
- (a) amplifying different dilutions of a nucleic acid and measuring their amplification in real-time;
  - (b) determining the cycle number at which amplification exceeds a threshold for each of the dilutions; and
  - (c) determining amplification efficiency of the nucleic acid as a function of concentration, from a non-linear, continuously differentiable function of the cycle number determined in step (b) that maps said cycle number to the

logarithm of concentration of the target nucleic acid, wherein the non-linear, continuously differentiable function is determined with a polynomial fit.

24. (Previously Added) The method of claim 23, wherein said polynomial fit is of the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> or 7<sup>th</sup> degree.
25. (Currently Amended) ~~The method of claim 20,~~ A method for determining amplification efficiency of a nucleic acid as a function of concentration comprising:
- (a) amplifying different dilutions of a nucleic acid and measuring their amplification in real-time;
  - (b) determining the cycle number at which amplification exceeds a threshold for each of the dilutions; and
  - (c) determining amplification efficiency of the nucleic acid as a function of concentration, from a non-linear, continuously differentiable function of the logarithm of concentration of nucleic acid that maps said logarithm of concentration to the cycle number determined in step (b), wherein the non-linear, continuously differentiable function is determined with a polynomial fit.
26. (Currently Amended) The method of claim ~~25~~ 26, wherein said polynomial fit is of the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> or 7<sup>th</sup> degree.
- 27.-30. (Canceled)
31. (Previously Amended) A method for quantifying a target nucleic acid relative to a reference nucleic acid in a sample to be analysed, wherein the sample to be analysed comprises the target nucleic acid and the reference nucleic acid, comprising:
- (a) amplifying different dilutions of the target nucleic acid and different dilutions of the reference nucleic acid and measuring amplification in real-time;
  - (b) determining the cycle number at which amplification exceeds a first threshold for each of the dilutions of step (a);
  - (c) generating a continuously differentiable target function,  $F_T$ , of target nucleic acid cycle number and a continuously differentiable reference function,  $F_R$ , of reference nucleic acid cycle number wherein:

$F_T$  maps the cycle numbers determined in step (b) for the dilutions of the target nucleic acid to the logarithm of concentration of the target nucleic acid and

$F_R$  maps the cycle numbers determined in step (b) for the dilutions of the reference nucleic acid to the logarithm of concentration of the reference nucleic acid;

- (d) amplifying the target nucleic acid and the reference nucleic acid in the sample to be analysed under similar amplification conditions and measuring amplification in real-time;
- (e) amplifying a calibrator sample and measuring amplification in real-time, wherein the calibrator sample comprises the target and reference nucleic acids in a known concentration ratio;
- (f) determining  $F_T(\text{Cp-Tar})$ ,  $F_R(\text{Cp-Ref})$ ,  $F_T(\text{Cp-Tar}_{\text{cal}})$  and  $F_R(\text{Cp-Ref}_{\text{cal}})$ , wherein:

$F_T(\text{Cp-Tar})$  is the value of the target function,  $F_T$ , at the cycle number (Cp-Tar) at which the amplification exceeds a second threshold for the target nucleic acid in step (d),

$F_R(\text{Cp-Ref})$  is the value of the reference function,  $F_R$ , at the cycle number (Cp-Ref) at which the amplification exceeds the second threshold for the reference nucleic acid in step (d),

$F_T(\text{Cp-Tar}_{\text{cal}})$  is the value of the target function,  $F_T$ , at the cycle number (Cp-Tar<sub>cal</sub>) at which the amplification exceeds the second threshold for the target nucleic acid in step (e) and

$F_R(\text{Cp-Ref}_{\text{cal}})$  is the value of the reference function,  $F_R$ , at the cycle number (Cp-Ref<sub>cal</sub>) at which the amplification exceeds the second threshold for the reference nucleic acid in step (e); and

- (g) quantifying the amount of target nucleic acid relative to the reference nucleic acid, wherein, the relative amount is:

$$\frac{F_T(\text{Cp-Tar}) / F_R(\text{Cp-Ref})}{F_T(\text{Cp-Tar}_{\text{cal}}) / F_R(\text{Cp-Ref}_{\text{cal}})}$$

32. (Previously Added) The method of claim 31, wherein the continuously differentiable function is determined with a polynomial fit.
33. (Previously Added) The method of claim 32, wherein said polynomial fit is of the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> or 7<sup>th</sup> degree.
34. (Previously Added) The method of claim 31, wherein the amplified nucleic acid is detected with at least one fluorescently labeled hybridization probe.
35. (Previously Added) The method of claim 34, wherein the amplified nucleic acid is detected with FRET hybridization probes, molecular beacons, or TAQMAN<sup>®</sup> probes.
36. (Previously Added) The method of claim 31, wherein the amplified nucleic acid is detected with a DNA-binding dye.
37. (Previously Added) The method of claim 36, wherein said DNA-binding dye is SYBR<sup>®</sup> Green I.
38. (New) A method for absolute quantification of a target nucleic acid in a sample comprising the steps of:
  - (a) determining the amplification efficiencies of the target nucleic acid and of an internal or external standard under defined amplification conditions by:
    - (i) preparing a dilution series of the target nucleic acid and a dilution series of the internal or external standard;
    - (ii) amplifying the target nucleic acid and the internal or external standard under defined reaction conditions and measuring the amplification in real-time;
    - (iii) setting a defined signal threshold value;
    - (iv) determining, for each dilution of the target nucleic acid and for each dilution of the internal or external standard, the cycle number at which the signal threshold value is exceeded;
    - (v) determining a non-linear continuously differentiable function of the cycle number determined in step (iv) as a function of the logarithm of



- the copy number of target nucleic acid and the internal or external standard used for the amplification; and
- (vi) calculating the amplification efficiency of the target nucleic acid and the internal or external standard from said non-linear continuously differentiable function;
- (b) amplifying the target nucleic acid contained in the sample and the internal or external standard under said defined reaction conditions;
  - (c) measuring the amplification of the target nucleic acid and that of the internal or external standard in real time; and
  - (d) calculating the original copy number in the sample by correcting the copy number derived from step c) with the amplification efficiencies determined in step a).
39. (New) A method for quantification of a target nucleic acid in a sample relative to a reference nucleic acid comprising the steps of:
- (a) determining the amplification efficiencies of the target nucleic acid and of the reference nucleic acid under defined amplification conditions by:
    - (i) preparing a dilution series of the target nucleic acid and a dilution series of the reference nucleic acid;
    - (ii) amplifying the target nucleic acid and the reference nucleic acid under defined reaction conditions and measuring the amplification in real-time;
    - (iii) setting a defined signal threshold value;
    - (iv) determining, for each dilution of the target nucleic acid and for each dilution of reference nucleic acid, the cycle number at which the signal threshold value is exceeded;
    - (v) determining a non-linear continuously differentiable function of the cycle number determined in step (iv) as a function of the logarithm of the copy number of target nucleic acid and the reference nucleic acid used for the amplification; and
    - (vi) calculating the amplification efficiency of the target nucleic acid and the reference nucleic acid from said non-linear continuously differentiable function;

- (b) amplifying the target nucleic acid contained in the sample as well as the reference nucleic acid contained in the sample under said defined amplification conditions;
- (c) measuring the amplification of the target nucleic acid and that of the reference nucleic acid in real time; and
- (d) calculating the original ratio of target nucleic acid and reference nucleic acid in the sample by correcting the ratio derived from step c) with the amplification efficiencies determined in step a).